

Attorney Docket No. P66602US0  
Application No. 09/841,157

**Amendments to the claims:**

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of claims:

Claims 1-14 (cancelled)

Claim 15. (new) A method for determining the similarity at the genome level between a target organism and a reference organism comprising the steps of:

a) preparing one or more of double-stranded DNA fragments by random PCR using, as a template, genome DNA of the target organism,

b) subjecting said double-stranded DNA fragments prepared in step a) to temperature gradient gel electrophoresis (TGGE) or denaturant gradient gel electrophoresis (DGGE), wherein an internal reference double-stranded DNA prepared in advance, of which the melting initiation point and the mobility transition end point are known, is co-migrated with the double-stranded DNA fragments,

c) assigning each melting initiation point and/or each mobility transition end point of the double-stranded DNA fragments prepared in step a) and both the melting initiation point and the mobility transition end point of the internal reference double-stranded DNA co-migrated from an electrophoretic pattern obtained in step b),

d) normalizing each coordinate of melting initiation point and/or mobility transition end point of the double-stranded DNA fragments using those of the melting initiation point and mobility

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transition end point of the internal reference double-stranded DNA to eliminate experimental fluctuations and generate species identification dots,

e) calculating PaSS (Pattern Similarity Score) defined by Eq.1 below:

$$\text{PaSS} = 1 - \{ \sum \gamma(i) \} / n \quad (1)$$

where ' $\sum$ ' denotes the summation over  $i = 1$  to  $n$  (where  $n$  is the number of the species identification dots used for the calculation), and  $\gamma(i)$  is expressed by Eq.2 below:

$$\gamma(i) = 2 \times |V_{1i} - V_{0i}| / (|V_{1i}| + |V_{0i}|) \quad (2)$$

where  $V_{0i}$  represents position vector of the  $i$ -th species identification dots of the reference organism and  $V_{1i}$  represents position vector of the  $i$ -th species identification dots of the target organism;

using species identification dots obtained in step d) and species identification dots of the reference organism to determine the similarity at the genome level between the target organism and the reference organism, wherein the species identification dots of the reference organism is separately obtained by a method in which steps a) to d) are carried out under the same conditions.

Claim 16. (new) A method for identifying a reference organism closest at the genome level to a target organism comprising the steps of:

a) preparing one or more of double-stranded DNA fragments by random PCR using, as a template, genome DNA of the target organism,

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b) subjecting said double-stranded DNA fragments prepared in step a) to temperature gradient gel electrophoresis (TGGE) or denaturant gradient gel electrophoresis (DGGE), wherein an internal reference double-stranded DNA prepared in advance, of which the melting initiation point and the mobility transition end point are known, is co-migrated with the double-stranded DNA fragments,

c) assigning each melting initiation point and/or each mobility transition end points of the double-stranded DNA fragments prepared in step a) and both the melting initiation point and the mobility transition end point of the internal reference double-stranded DNA co-migrated from an electrophoretic pattern obtained in step b),

d) normalizing each coordinate of melting initiation point and/or mobility transition end point of the double-stranded DNA fragments using those of the melting initiation point and/or mobility transition end point of the internal reference double-stranded DNA to eliminate experimental fluctuations and generate species identification dots,

e) calculating PaSS (Pattern Similarity Score) defined by Eq.1 below:

$$\text{PaSS} = 1 - \{ \sum \gamma(i) \} / n \quad (1)$$

where ' $\sum$ ' denotes the summation over  $i = 1$  to  $n$  (where  $n$  is the number of the species identification dots used for the calculation), and  $\gamma(i)$  is expressed by Eq.2 below:

$$\gamma(i) = 2 \times |V_{1i} - V_{0i}| / (|V_{1i}| + |V_{0i}|) \quad (2)$$

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where  $V_{0i}$  represents position vector of the  $i$ -th species identification dots of the reference organism and  $V_{1i}$  represents position vector of the  $i$ -th species identification dots of the target organism;

using species identification dots obtained in step d) and species identification dots of a reference organism deposited in a database in which species identification dots of organisms separately determined by a method in which steps a) to d) are carried out under the same conditions are registered, and

f) repeating calculation of PaSS of step e) with altering the reference organism until the maximum PaSS is reached to identify the reference organism closest at the genome level to the target organism.

Claim 17. (new) A method for determining the similarity at the genome level between a target organism and a reference organism comprising the steps of:

a) preparing one or more of double-stranded DNA fragments by random PCR using, as a template, genome DNA of the target organism,

b) subjecting said double-stranded DNA fragments prepared in step a) to temperature gradient gel electrophoresis (TGGE) or denaturant gradient gel electrophoresis (DGGE), wherein an internal reference double-stranded DNA prepared in advance, of which the melting initiation point

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and the mobility transition end point are known, is co-migrated with the double-stranded DNA fragments,

c) assigning each melting initiation point and/or each mobility transition end point of the double-stranded DNA fragments prepared in step a) and both the melting initiation point and the mobility transition end point of the internal reference double-stranded DNA co-migrated from an electrophoretic pattern obtained in step b),

d) normalizing each coordinate of melting initiation point and/or mobility transition end point of the double-stranded DNA fragments using those of the melting initiation point and mobility transition end point of the internal reference double-stranded DNA to eliminate experimental fluctuations and generate species identification dots,

e) calculating genome semi-distance by the formula

$$(1 - \text{Pass}) / \text{Pass}$$

to determine the similarity at the genome level between the target organism and the reference organism, PaSS (Pattern Similarity Score) defined by Eq.1 below:

$$\text{PaSS} = 1 - \{ \sum \gamma(i) \} / n \quad (1)$$

where ' $\sum$ ' denotes the summation over  $i = 1$  to  $n$  (where  $n$  is the number of the species identification dots used for the calculation), and  $\gamma(i)$  is expressed by Eq.2 below:

$$\gamma(i) = 2 \times |V_{1i} - V_{0i}| / (|V_{1i}| + |V_{0i}|) \quad (2)$$

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where  $V_{0i}$  represents position vector of the  $i$ -th species identification dots of the reference organism and  $V_{1i}$  represents position vector of the  $i$ -th species identification dots of the target organism;

using species identification dots obtained in step d) and species identification dots of the reference organism to determine the similarity at the genome level between the target organism and the reference organism, wherein the species identification dots of the reference organism is separately obtained by a method in which steps a) to d) are carried out under the same conditions.

Claim 18. (new) A method for identifying a reference organism closest at the genome level to a target organism comprising the steps of:

a) preparing one or more of double-stranded DNA fragments by random PCR using, as a template, genome DNA of the target organism,

b) subjecting said double-stranded DNA fragments prepared in step a) to temperature gradient gel electrophoresis (TGGE) or denaturant gradient gel electrophoresis (DGGE), wherein an internal reference double-stranded DNA prepared in advance, of which the melting initiation point and the mobility transition end point are known, is co-migrated with the double-stranded DNA fragments,

c) assigning each melting initiation point and/or each mobility transition end points of the double-stranded DNA fragments prepared in step a) and both the melting initiation point and the

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mobility transition end point of the internal reference double-stranded DNA co-migrated from an electrophoretic pattern obtained in step b),

d) normalizing each coordinate of melting initiation point and/or mobility transition end point of the double-stranded DNA fragments using those of the melting initiation point and/or mobility transition end point of the internal reference double-stranded DNA to eliminate experimental fluctuations and generate species identification dots,

e) calculating genome semi-distance by the formula

$$(1 - \text{Pass}) / \text{Pass}$$

to determine the similarity at the genome level between the target organism and the reference organism, PaSS (Pattern Similarity Score) <sup>being</sup> defined by Eq. 1 below:

$$\text{PaSS} = 1 - \{ \sum \gamma(i) \} / n \quad (1)$$

where ' $\sum$ ' denotes the summation over  $i = 1$  to  $n$  (where  $n$  is the number of the species identification dots used for the calculation), and  $\gamma(i)$  is expressed by Eq. 2 below:

$$\gamma(i) = 2 \times |V_{1i} - V_{0i}| / (|V_{1i}| + |V_{0i}|) \quad (2)$$

where  $V_{0i}$  represents position vector of the  $i$ -th species identification dots of the reference organism and  $V_{1i}$  represents position vector of the  $i$ -th species identification dots of the target organism;

using species identification dots obtained in step d) and species identification dots of a reference organism deposited in a database in which species identification dots of organisms separately

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determined by a method in which steps a) to d) are carried out under the same conditions are registered, and

f) repeating calculation of genome semi-distance of step e) with altering the reference organism until the maximum genome semi-distance is reached to identify the reference organism closest at the genome level to the target organism.

Claim 19. (new) A method according to claim 15, wherein said standard DNA is SEQ ID NO: 1 or SEQ ID NO: 2.

Claim 20. (new) A method according to claim 15, wherein said identification of an organism is the species identification or homology identification of an organism.

Claim 21. (new) A method according to claim 15, wherein in step a), a primer or nucleotide labeled with a fluorescent marker is used for said random PCR to amplify DNA fragments with a fluorescent marker and a fluorescence labeled DNA is used as the standard DNA, and in step c), said extraction from the featuring points is carried out by image processing using the fluorescent markers carried by the DNAs.

Claim 22. (new) A method according to claim 15, wherein the featuring points which are obtained in step c) are expressed by the coordinates of the temperature axis and the mobility axis in the case



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of temperature gradient gel electrophoresis (TGGE), and by the coordinates of the denaturant concentration axis and the mobility axis in the case of denaturant gradient gel electrophoresis (DGGE).

Claim 23. (new) A method according to claim 15, wherein said organism is a microorganism.

Claim 24. (new) A method according to claim 16, wherein said standard DNA is SEQ ID NO: 1 or SEQ ID NO: 2.

Claim 25. (new) A method according to claim 16, wherein said identification of an organism is the species identification or homology identification of an organism.

Claim 26. (new) A method according to claim 16, wherein in step a), a primer or nucleotide labeled with a fluorescent marker is used for said random PCR to amplify DNA fragments with a fluorescent marker and a fluorescence labeled DNA is used as the standard DNA, and in step c), said extraction from the featuring points is carried out by image processing using the fluorescent markers carried by the DNAs.

Claim 27. (new) A method according to claim 16, wherein the featuring points which are obtained in step c) are expressed by the coordinates of the temperature axis and the mobility axis in the case

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of temperature gradient gel electrophoresis (TGGE), and by the coordinates of the denaturant concentration axis and the mobility axis in the case of denaturant gradient gel electrophoresis (DGGE).

Claim 28. (new) A method according to claim 16, wherein said organism is a microorganism.

Claim 29. (new) A method according to claim 17, wherein said standard DNA is SEQ ID NO: 1 or SEQ ID NO: 2.

Claim 30. (new) A method according to claim 17, wherein said identification of an organism is the species identification or homology identification of an organism.

Claim 31. (new) A method according to claim 17, wherein in step a), a primer or nucleotide labeled with a fluorescent marker is used for said random PCR to amplify DNA fragments with a fluorescent marker and a fluorescence labeled DNA is used as the standard DNA, and in step c), said extraction from the featuring points is carried out by image processing using the fluorescent markers carried by the DNAs.

Claim 32. (new) A method according to claim 17, wherein the featuring points which are obtained in step c) are expressed by the coordinates of the temperature axis and the mobility axis in the case

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of temperature gradient gel electrophoresis (TGGE), and by the coordinates of the denaturant concentration axis and the mobility axis in the case of denaturant gradient gel electrophoresis (DGGE).

Claim 33. (new) A method according to claim 17, wherein said organism is a microorganism.

Claim 34. (new) A method according to claim 18, wherein said standard DNA is SEQ ID NO: 1 or SEQ ID NO: 2.

Claim 35. (new) A method according to claim 18, wherein said identification of an organism is the species identification or homology identification of an organism.

Claim 36. (new) A method according to claim 18, wherein in step a), a primer or nucleotide labeled with a fluorescent marker is used for said random PCR to amplify DNA fragments with a fluorescent marker and a fluorescence labeled DNA is used as the standard DNA, and in step c), said extraction from the featuring points is carried out by image processing using the fluorescent markers carried by the DNAs.

Claim 37. (new) A method according to claim 18, wherein the featuring points which are obtained in step c) are expressed by the coordinates of the temperature axis and the mobility axis in the case

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of temperature gradient gel electrophoresis (TGGE), and by the coordinates of the denaturant concentration axis and the mobility axis in the case of denaturant gradient gel electrophoresis (DGGE).

Claim 38. (new) A method according to claim 18, wherein said organism is a microorganism.